Iranian Journal of Basic Medical Sciences

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IJBMS

Frequencies of HLA-A, B and DRB1 alleles in a large normal population living in the city of Mashhad, Northeastern Iran

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Sep 27, 2016
Accepted: May 25, 2017

Keywords:
Allele frequency
HLA polymorphism
Human leukocyte antigen
Iranian population
Major histocompatibility - Complex
Polymerase chain reaction

ABSTRACT

Objective(s): The population in Iran is a genetic admixture of the ancestral Aryan and other populations neighboring Iran. Different ethnic groups in Iran show wide regional distributions for many human leukocyte antigen (HLA) alleles. Therefore, it is necessary and sensible to study the differences in HLA allele distribution in different area. We studied the HLA class I and II allele frequencies in a large unrelated healthy Iranian population from Mashhad in the Northeast region.

Materials and Methods: Five hundred unrelated healthy adult individuals borne and living in Mashhad, Northeast of Iran, were genotyped for HLA-A, B and HLA-DRB1 alleles using PCR with low resolution sequence specific primers (SSP-PCR) technique.

Results: A total of 14 HLA-A, 24 HLA-B and 10 HLA-DRB1 alleles were spread throughout the studied population with distinct allele frequencies. At the HLA-A locus, HLA-A*02 was found to be the most frequent allele, with a frequency of 20.9%. The most common HLA-B alleles was B*35 (16.4%). The two most common observed alleles in HLA class II alleles were DRB1*15 (20.0%) followed by DRB1*13 (16.2%).

Conclusion: This study is the first on the HLA class I and II allele frequencies in Northeastern Iranian population living in Mashhad. Distribution of HLA-A and B loci showed some similarities with those of other Iranians. Some difference in HLA-DRB1 polymorphisms however was observed. Considering the highly mixed population of Mashhad, the finding was not unexpected.

► *Please cite this article as:*
Esmaeili AR, Zamani Taghizadeh Rabe Sh, Mahmoudi M, Rastin M. Frequencies of HLA-A, B and DRB1 alleles in a large normal population living in the city of Mashhad, Northeastern Iran. Iran J Basic Med Sci 2017; 20:940-943. doi: 10.22038/IJBMS.2017.9117

Introduction

The human leukocyte antigen (HLA) class I and II are the most polymorphic gene clusters in the human genome. They play an important role in presenting antigenic peptides to $CD4^+$ and $CD8^+$ T lymphocytes that induce immune responses. HLA cluster includes six classical genes and encodes at least 132 proteins, which have multiple functions in the regulation of immune responses (1).

Remarkable extent of the allelic diversity at these loci has been revealed over the past decades by molecular genetic analyses using various techniques. Sequence polymorphism in the antigen binding domains of the HLA molecules defines the repertoire of peptides that can be presented and are able to regulate immune responses (2). According to the ImMunoGeneTics (IMGT)/HLA database (http://www.ebi.ac.uk/imgt/hla/), 16429 HLA alleles have been introduced from different populations throughout the world (3).

In spite of the wide use of various types of genetic markers, allelic frequency distributions of the HLA class I and II loci and their linkage disequilibrium patterns continue to be regarded as valuable markers for the determination of genetic relatedness between different populations (4). High diversity of the HLA alleles and haplotypes is widely used to explore and validate the origins and migration of various human populations (5-7). In addition, some populations have specific frequencies of HLA-A, B, C and DR alleles that are important to distinguish the genetic structure between closely related ethnic groups. Moreover, knowledge of this genetic system is helpful in organ and hematopoietic stem cell transplantation, vaccine development, anthropological studies, and disease association (8).

Iran is one of the oldest countries in the world and has an ancient history of about 3500 years. Over the past times, Iran has seen many invasions and has had several periods of expansion. Iran is located in south-western Asia, with a population of over 75 million individuals and a vast common border with neighboring countries. Iran has borders with the Republic of Armenia, Azerbaijan, Turkmenistan,

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Turkey, Iraq, Afghanistan and Pakistan. Iranian ancestors were of Caucasian origin, but Arab and subsequent Mongol and Tatar invasions, each left its own imprint on the development of different populations in this country (9). Therefore, in the phylogenetic tree, Iranians are considered as a highly mixed population of Caucasian origin. Because of several invasions and immigrations from neighboring countries, there exist diverse ethnic and tribal groups such as Persian, Turk, Kurd, Arab, Turkmen, Baloch and Lur in Iran (10-13).

Ethnic diversity of Iranian population had a strong influence on the HLA frequencies in different regions. In previous studies, HLA allele frequencies have been reported for different regions of Iran (14-19). Although Northeast of Iran is of considerable interest, due to its vast territories of exceptional variety, limited information is available on the distribution of HLA alleles from this region.

Mashhad is located in the Northeast of Iran, and is the second most crowded city in the country. It is close to the borders of Turkmenistan and Afghanistan, and is located along the Silk Road. Over the history, the city has experienced some invasions, which brought about great changes in its ethnic composition. Most inhabitants in Mashhad are Persian. However, there also exists a great mixed population in the city.

Therefore, the present study was undertaken to establish the Iranian HLA class I and II gene profile on a large population sample living in Mashhad, Khorasan Razavi province, Northeast of Iran.

Materials and Methods

Population and sample preparation

A total number of 500 healthy adults (248 men with a mean age of 35.26±14.79 years and 252 women with a mean age of 33.13±15.01 years), living in the city of Mashhad, Khorasan Razavi province, Northeast of Iran participated in this study. Intravenous blood samples (5–10 ml) were drawn and collected in EDTA tubes. Peripheral blood mononuclear cells (PBMCs) were separated from peripheral blood by density gradient centrifugation over Ficoll-hypaque (Biosera, UK) and were washed three times with Hank's buffered solution (HBSS).

HLA class I and II typing using SSP-PCR method

Genomic DNA was extracted from PBMCs using a QIAamp Blood Kit (QIAGEN, GmbH) according to the manufacturer's protocol. DNA samples were quantified spectrophotometrically at 260 nm and then stored at -70 °C. The HLA-A, B and HLA-DRB1 alleles of each sample were determined using a low resolution sequence specific primer polymerase chain reaction (SSP-PCR) technique (Inno-Train, Germany). SSP-PCR is a molecular HLA typing method that is widely used for the determination of HLA class I and II alleles. The PCR reactions were carried out in 10 µl volumes. Samples were first denatured at 96 °C for 2 min, followed by 10 cycles of 96 °C 15 sec, 65 °C 60 sec and 20 cycles of 96 °C 15 sec, 61 °C 50 sec and 72 °C 30 sec. The products were then run on electrophoresis through an agarose gel. Interpretation of the results was performed using kit manufacture's data sheets and software (SCORE Software).

Table 1. The gene frequencies (F %) of human leukocyte antigen (HLA)-A, B and HLA-DRB1 alleles in 500 Iranian individuals

HLA-A	F (%)	HLA-B	F (%)	HLA-DRB1	F (%)
A*01	11.5 %	B*07	4.4 %	DRB1*01	7.5 %
A*02	20.9 %	B*08	3.0 %	DRB1*03	8.7 %
A*03	11.1 %	B*13	6.0 %	DRB1*04	12.5 %
A*11	11.3 %	B*14	4.0 %	DRB1*07	15.0 %
A*23	5.2 %	B*15	2.95 %	DRB1*09	1.25 %
A*24	10.2 %	B*18	6.5 %	DRB1*11	15.0 %
A*26	6.6 %	B*27	1.8 %	DRB1*13	16.2 %
A*29	2.7 %	B*35	16.4 %	DRB1*14	2.5 %
A*30	5.5 %	B*37	1.45 %	DRB1*15	20.0 %
A*31	2.7 %	B*38	6.1 %	DRB1*16	1.25 %
A*32	3.3 %	B*39	2.2 %		
A*33	2.7 %	B*40	4.3 %		
A*68	5.7 %	B*41	5.1 %		
A*69	0.55 %	B*44	4.3 %		
		B*45	0.2 %		
		B*46	0.1 %		
		B*49	4.7%		
		B*50	5.4 %		
		B*51	9.2 %		
		B*52	3.8 %		
		B*53	1.45 %		
		B*55	3.6%		
		B*57	2.3 %		
		B*58	0.2 %		

Results

HLA class I and II allele frequencies

In the present study, we reported the results of HLA class I and II genotyping of 500 healthy adults living in the city of Mashhad, Northeastern Iran.

We detected a total number of 14 and 24 alleles in HLA-A and B loci, respectively. As presented in Table 1, the most frequent HLA class I alleles were A*02 (20.9%), A*01 (11.5%), B*35 (16.4%) and B*51 (9.2%), while, the least frequent alleles were A*69 (0.55%), and B*46 (0.1%). Among total HLA-class I alleles, the most prevalent allele in the studied subjects of Iranian population was HLA-A*02.

In HLA class II alleles, we identified 10 alleles in HLA-DRB1 loci (Table 1). The two most common HLA class II alleles were DRB1*15 (20.0%) followed by DRB1*13 (16.2%), and the least frequent alleles were DRB1*09 (1.25%) and DRB1*16 (1.25%).

Discussion

The distribution of HLA alleles and haplotypes varies significantly in different populations, which may widen our horizon on the evolution of HLA polymorphism as well as on the origin and migration of human populations. In the present study, we evaluated the allele frequency of HLA-A, B and DRB1 genes in a normal population living in the city of

Mashhad, Khorasan Razavi province, Northeastern Iran. This is the first comprehensive analysis of HLA class I and II genes in this population and the obtained results indicate extensive HLA diversity and some genetic similarities with other ethnic groups of Iran (14, 21, 22). In 500 individuals genotyped, we detected the HLA A*02 (20.9%), B*35 (16.4%), and DRB1*15 (20.0%) as the most frequent alleles for HLA class I and II respectively. In HLA-A locus, A*02, A*01, A*11, A*03 and A*24 represented 65% of the gene frequencies; in HLA-B locus, B*35, B*51, B*18, B*38, B*50 and B*41 accounted for more than 54 percent of the gene frequencies, while in HLA-DRB1 locus, DRB1*15, DRB1*13, DRB1*11, DRB1*07 and DRB1*04 represented 78% of gene frequencies.

Some investigators have previously characterized the HLA class I and II polymorphisms in different regions of Iran (14-19, 21-25). In agreement with the results of the present study in most of the surveys, HLA-A*02 showed the highest prevalence in HLA-A allele, whereas in HLA-B locus, B*35 was the most frequent one (14, 16, 17 21, 24).

In contrast to HLA class I alleles, studies concerning the distribution of HLA-DRB1 in Iranian people showed some differences in this locus. We identified DRB1*15 as the most prevalent allele, although in several studies HLA-DRB1*11 was reported as the most prevalent one (15, 17-18, 21, 23-27), while some others reported DR2 (28), and DRB1*07 (29) as more frequent alleles. Interestingly in most of the studies DRB1*15 was reported as the second most prevalent HLA-DRB1 allele in Iranian population (18, 19, 21, 23, 27).

Iranians are considered to have Caucasians origin, but diverse ethnic and tribal groups such as Persian, Turk, Kurd, Arab, Turkmen, Baloch and Lur live in Iran (25, 30). Some authors studied the allele frequency of HLA-DRB1 alleles in different ethnic groups living in Iran, and showed evident diversity in the distribution of DRB1 in these populations. In the Baloch ethnic group living in the southeast of Iran, DRB1*03 (16, 25) and DR2 (17), in Famoori Arabs living in Fars province in south of Iran, DRB1*03 (25) and in Khuzestani Arabs living in Ahvaz in southwest of the country, DRB1*07 (25) were reported as the most common alleles, while in Zoroastrian ethnic groups living in Yazd in the center of Iran, DRB1*07 (25) showed the highest frequency.

Mashhad is located in the Northeast of Iran and has a very mixed population in comparison to the other parts of Iran. Mashhad is a city with many immigrants. In addition, millions of travelers and pilgrims visit the city annually from both inside and outside of the country (from Afghanistan, Pakistan, Azerbaijan, Turkmenistan and Arabic countries from Middle-East). Considering the highly mixed population living in Mashhad, the diversity in the distribution of HLA-DRB1 alleles, which we detected, was not unexpected.

In our study, HLA-DRB1*08 and DRB1*12 alleles were not detected in any of the participants, and DRB1*09 and DRB1*16 showed frequency of 1.25%. Interestingly in almost all previously published studies, in agreement with our results, DRB1*08, DRB1*09 and DRB1*12 were reported as the alleles with the lowest frequency in Iranian population (15, 16, 18, 19, 21, 23-25, 27, 29).

In general, according to the results of this study and those of the others, it can be concluded that Iranian people are highly mixed population, and although all different ethnic inhabitants have some shared relatedness or origin, there exists some diversity in the distribution of HLA-DRB1, which requires further investigation.

Nonetheless, this study was not without limitations. Participants of the study, for instance, were all borne and lived in Mashhad, but we did not have access to any data regarding the preceding generation of the study participants.

Conclusion

On the basis of our results, Iranian individuals living in Mashhad demonstrated some genetic similarities with the other Iranians in HLA Class I loci. Nevertheless, considering the highly mixed population living in Mashhad, some differences in HLA-DRB1 polymorphisms that we detected were to be expected. Further differential studies in each subpopulations living in Mashhad and other parts of the province will provide more information about the impact of immigration, past history and environmental forces on the genetic makeup of this population.

Acknowledgment

This study was supported by grant number 88374 from vice president for research, Mashhad University of Medical Sciences.

References

1. Shiina T, Hosomichi K, Inoko H, Kulski JK. The HLA genomic loci map: expression, interaction, diversity and disease. J Hum Genet 2009: 54: 15–39.
2. Parham P, Ohta T. Population biology of antigen presentation by MHC class I molecules. Science 1996; 272: 67–74.
3. Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SGE. The IMGT/HLA database. Nucleic Acids Res 2011; 39: 1171–1176.
4. Pedron B, Yakouben K, Guerin V, Borsali E, Auvrignon A, Landman J. HLA alleles and haplotypes in French North African immigrants. Hum Immunol 2006; 67: 540–550.
5. Ferrer A, Nazabal M, Companioni O, Fernandez de Cossio ME, Camacho H, Cintado A. HLA class I polymorphism in the Cuban population. Hum Immunol 2007; 68: 918–927.
6. Arnaiz-Villena A, Gomez-Casado E, Martinez-Laso J. Population genetic relationships between Mediterranean

populations determined by HLA allele distribution and a historic perspective. Tissue Antigens 2002; 60: 111–121.
7. Vargas-Alarcon G, Moscoso J, Martinez-Laso J. Origin of Mexican Nahuas (Aztecs) according to HLA genes and their relationships with worldwide populations. Mol Immunol 2007; 44: 747–755.
8. Tokunaga K, Imanishi T, Takahashi K, Juji T. On the origin and dispersal of East Asian populations as viewed from HLA haplotypes. Oxford: Oxford University, 1996.
9. Momeni DA. In: The Population of Iran: A Dynamic Analysis. Tehran: Pahlavi University Publication, 1975.
10. Boulonois I. In: The Silk Road. London: Allen & Unwin, 1966.
11. Al-Taie A. Ethnic Identity Crisis in Iran. Tehran: Shadegan publication 2000: 167–70.
12. Calafell F. Classifying humans. Nat Genet 2003: 33: 435–6.
13. Lin M, Chu CC, Chang SL. The origin of Minnan and Hakka, the so-called ''Taiwanese'', inferred by HLA study. Tissue Antigens 2001: 57: 192–9.
14. Ghashghaie A, Alimoghaddam K, Ostadali MR, Ghaffari H, Khansari L, Sadraee M, Mirrasekhian E, Mohyedin I, Raoofi F, Noori Z, Yaghmaian H, Eshaghi H, Ghavamzadeh A. Allele frequencies of HLA-class-I loci in normal Iranian population. I J HOSCR 2009 3: 18-20.
15. Mohammadi M, Rastin M, Rafatpanah H, Abdoli Sereshki H, Zahedi MJ, Nikpoor AR, Baneshi MR, Hayatbakhsh MM. Association of HLA-DRB1 Alleles with Ulcerative Colitis in the City of Kerman, South Eastern Iran. Iran J Asthma Allergy Immunol 2015; 14: 306-312.
16. Farjadian S, Naruse T, Kawata H, Ghaderi A, Bahram S, Inoko H. Molecular analysis of HLA allele frequencies and haplotypes in Baloch of Iran compared with related populations of Pakistan. Tissue Antigens 2004: 64: 581–587.
17. Khazaei HA, Aghamohammadi A, Rezaei N, Nikbin B, Khosravi MA, Miri Moghaddam I. Major histocompatibility complex class I and II antigens frequencies in Baloch ethnic group living in the southeast region of Iran. Transplantation Proceedings 2004; 36: 1302–1304.
18. Amirzargar A, Mytilineos J, Farjadian Sh, Doroudchi M, Scherer S, Opelz G, Ghaderi A. Human leukocyte antigen class II allele frequencies and haplotype association in Iranian normal population. Human Immunology 2001; 62: 1234–1238.
19. Yari F, Sobhani M, Sabaghi F, Zaman-Vaziri M, Bagheri N, Talebian A. Frequencies of HLA-DRB1 in Iranian normal population and in patients with acute lymphoblastic leukemia. Arch Med Res 2008; 39: 205-208.
20. Hong W, Chen S, Shao H, Fu Y, Hu Z, Xu A. HLA class I polymorphism in Mongolian and Hui ethnic groups from Northern China. Human Immunology 2007; 68: 439-448.
21. Shaiegan M, Yari F, Abolghasemi H, Bagheri N, Paridar M, Heidari A, Kaskani Sh , Sabaghi F, Dikloo F, Zaman-Vaziri M, Sobhani M, Riazi S, Karimi M. Allele Frequencies of HLA-A, B and DRB1 among People of Fars Ethnicity Living in Tehran. IJBC 2011;4 : 55-59.
22. Ghashghaie A, Ghaffari SH, Alimoghadam K, Ghavamzadeh A. Typing of HLA Class I by Polymerase Chain Reaction-Sequence Specific Oligonucleotide Primer (PCR-SSOP) Technique in Iranian Cord Blood Donors. Iran.J.Immunol. 2006; 3: 95-98.
23- Sarafnejad A, Khosravi F, Alimoghadam K, Dianat S, Ansaripour B, Moradi B, Dorkhosh SH, Amirzargar A. HLA Class II Allele and Haplotype Frequencies in Iranian Patients with Acute Myelogenous Leukemia and Control Group 2006; 5: 115-119.
24. Sayad A, Akbari MT, Mehdizadeh M, Movafagh A, Hajifathali A. The Association of HLA-Class I and Class II with Hodgkin's Lymphoma in Iranian Patients. Biomed Res Int 2014; 2014: 231236. Published online 2014 May 21. doi: 10.1155/2014/231236 PMCID.
25. Farjadian SH, Ota M, nook H, Ghaderi A. The genetic relationship among Iranian ethnic groups: an anthropological view based on HLA class II gene polymorphism. Mol Biol Rep 2009; 36: 1943- 1950.
26. Mahmoodi M, Nahvi H, Mahmoudi M, Kasaian A, Mohagheghi MA, Divsalar K, Nahavandian B, Jafari A, Ansarpour B, Moradi B, Aghamohammadi A, Amirzargar A. HLA-DRB1,-DQA1 and -DQB1 Allele and Haplotype Frequencies in Female Patients with Early Onset Breast Cancer. Pathol Oncol Res 2012; 18: 49-55.
27. Khosravi F, Amirzargar A, Sarafnejad A, Nickanam MH, Alimoghadam K, Dianat S, Solgi Gh, Nikbin B. HLA Class II Allele and Haplotype Frequencies in Iranian Patients with Leukemia. Iran J Allergy Asthma Immunol 2007; 6: 137-142.
28. Amanzadel A, Shokrgozar MA, Samadi-Bahrami Z, Aghamohammadi A, Shokri F. Frequency Analysis of HLA Antigens in Iranian Patients with Common Variable Immunodeficiency. Arch Iranian Med 2003; 6: 16-22.
29. Amanzadeh A, Amirzargar A, Mohseni N, Arjang Z, Aghamahammadi A, Shokrgozar MA, Shokri F. Association of HLA-DRB1, DQA1 and DQB1 Alleles and Haplotypes with Common Variable Immunodeficiency in Iranian Patients. Avicenna J Med Biotech 2012; 4: 103-112.
30. Farjadian Sh, Ghaderi A. HLA class II Genetic Diversity in Arabs and Jews of Iran. Irn J Immunol 2007; 4: 85-93.